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Original communication

Clinical forensic sample collection techniques following consensual intercourse in volunteers – Cervical canal brush compared to conventional swabs

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ABSTRACT

The purpose of the research was to evaluate gynecological evidence collection techniques; the benefit of cervical canal brush sample compared to vaginal fornix and cervical swab samples and the time frame for detecting Y-chromosomal material QiAmp DNA Mini Kit® and Quantifiler Y Human Male DNA Quantification Kit® in adult volunteers following consensual intercourse.

Eighty-four adult female volunteers following consensual intercourse were recruited for the study. By combining all sample collecting techniques, 81.0% of the volunteers were Y-DNA positive. Up to 60 h the conventional swab sampling techniques detected more Y-DNA positive samples when compared to the brush technique. However, after 60 h, the cervical canal brush sample technique showed its benefit by detecting 27.3% (6/22) of Y-DNA positive samples, which were Y-DNA negative in both conventional swab sampling techniques. By combining swab and brush techniques, 75% of the volunteers were still Y-DNA positive in 72–144 post-coital hours. The rate of measurable Y-DNA decreased approximately 3% per hour. Despite reported consensual intercourse, 6.8% (3/44) of volunteers were Y-DNA negative within 48 h. Y-DNA was not detected after 144 post-coital hours (6 days).

In conclusion, the brush as a forensic evidence collection method may provide additional biological trace evidence from the cervical canal, although the best biological trace evidence collection can be obtained by combining all three sampling techniques. The time frame for gynecological forensic evidence sample collection should be considered to be at least a week if sexual violence is suspected.

1. Introduction

In suspected sexual assault cases the purpose of forensic biological evidence collection is to detect spermatozoa to indicate sexual contact, and to detect semen for DNA identification.

After vaginal penile penetration, absence of spermatozoa in microscopy may result from the lack of ejaculation, or due to vasectomy, low count of spermatozoa in the seminal fluid, or loss of semen from the vagina before evidence collection. A recent study detected spermatozoa in 88% (28/32) cases who reported ejaculation during the studied consensual intercourse.[1] In police reported victims of sexual violence, spermatozoa were detected in 35.8% (45/151) females by microscopy.[2]

Detecting male DNA from the vaginal fornix or uterine cervix may result from alleged sexual assault, previous consensual intercourse, contamination, or a rare XY syndrome in a female.[3] In a previous study of alleged sexual assault victims with negative cytology for spermatozoa, Y-DNA was detected in swabs in 43.9% (25/57) when vaginal penetration was the suspected assault type.[4]

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The cut-off time frame for collecting forensic genital samples has routinely been three days in alleged sexual assaults in Finland. The international recommendation for routine evidence collection from the vaginal fornix and cervical canal varies from 3 to 10 days.[5] To our knowledge, the longest reported post-coital time interval for the spermatozoa to be present in the cervical smear is 12 days and 9 days in the vagina.[6]

The aim of the study was to evaluate forensic collection methods among volunteers by comparing the traditional, vaginal fornix and cervical canal swabs to cervical canal brush samples, and to evaluate the time frames for a measurable quantity of Y-chromosomal material. The study hypothesis was that the brush samples may provide additional biological evidence from the cervical canal as the brush attaches most of the cervical canal mucus to the brush when compared to traditional genital swab samples.

2. Material and methods

2.1. Participant characteristics

Female volunteers, comprising medical or laboratory students, hospital personnel or acquaintances, were invited to participate in a gynecological examination after consensual vaginal intercourse between May 2008 and December 2009 by either a personal invitation or by collective invitation after a lecture. 84 volunteers were included in the study. Two volunteers were excluded because of pregnancy. Most of the volunteers (91%) attended to another study comparing white light to UV-light in extra genital lesions.

The women were instructed to record the time of their last intercourse before the examination. The sample collection time was recorded. Medical histories were given by the volunteers using a form. Contraception, number of partners, last menstruation, parity, time of previous intercourse before the intercourse for the sample collection, frequency of intercourse in the past two weeks prior to the sample collection, use of lubricants, activities after the intercourse (wiping, washing, showering, going to sauna, urinating, defecating) were recorded.

2.2. Sample collection

Three samples were collected; cotton swabs (Invasive sterile Eurotubo® collection swab, Deltalab, Rubi, Spain) first from the vaginal fornix, following a cervical canal swab sample and a third sample from the cervical canal with a Papanicolaou (PAP) smear cervical brush (Gynobrush® Plus, Heinz Herenz, Hamburg, Germany) (Fig. 1). A cervical brush was spun in the cervical canal so that it would collect as much cervical mucus as possible. The swab was also spun in the cervical canal. One gynecologist collected all the samples.

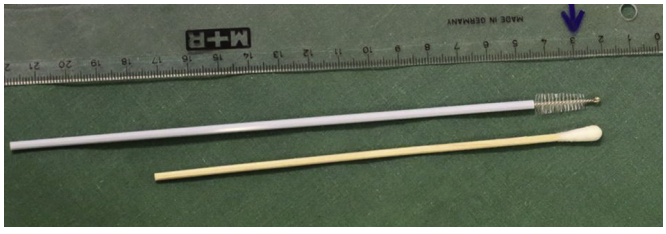

Fig. 1. Photograph of brush and swab.

2.3. Laboratory techniques

The cervical mucus was first mechanically removed from the brush by rubbing the brush against the wall of the microcentrifuge-tube which contained 400 μl of Phosphate-Buffered Saline (PBS). DNA from the swab and brush samples was extracted using QIAamp DNA Mini Kit® (Qiagen, Germany) with Buccal Swab Spin Protocol.

Quantifiler Y Human Male DNA Quantification Kit® (Applied Biosystems, USA) was used to quantify the total amount of amplifiable male DNA in a sample. Analysis was done with AbiPrism® 7000 HT Sequence Detection System (AppliedBiosystems, USA) according to instructions provided. A sample was considered positive if a measurable amount of DNA was detected.

2.4. Direct microscopy

Microscopic examinations were performed altogether in 62 (73.8%) volunteers. Immediate microscopy was performed on 39 out of 84 (46.4%) volunteers in a forensic laboratory. The following parameters were examined: volume, motility and sperm density. The density was categorized to no sperm, a few sperm (1–10/slide), a moderate amount (10–50/slide), and many (>50/slide). The examination was performed according to the laboratory manual WHO 1999 (WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction 1999). Each sample was suspended on a slide by mixing the sample with a small amount of a culture medium (5 μl). The covered preparation was examined under the microscope. All samples were examined by phase-contrast microscope (magnification of 10 × 20).

In 23 (27.4%) volunteers samples from swabs and from brushes were collected onto slides which were dried and dyed with semen stain identification (Kernechrot Picoindigocarmine Stain®). Spermatozoa were verified at 1000× magnification microscopy.

2.5. Contamination issues

All the collection, extraction and amplification steps were performed by the same female experimenters to avoid secondary Y-DNA contamination. The paper cover on the examination table was changed and the table with surrounding area was wiped with disposable disinfecting cloths between volunteers. Disposable powder free gloves were used and changed frequently to minimize the contamination risk.

2.6. Ethical considerations

The study protocol was approved by the Ethics Committee (#R08018) of Pirkanmaa Hospital District, Finland. A written consent was required for attendance to the study, for forensic laboratory analysis and for publication purposes. No data from the patient's medical records were collected. Full anonymity was secured in storage and laboratory analysis.

2.7. Statistical considerations

Results are presented as medians and ranges or as frequencies and percentages. Differences between collection methods were analyzed using the McNemar test. Positivity of different collection methods were analyzed by binary logistic regression using time from intercourse to medical examination, parity, contraception and post-coital activities as predictor variables. Results are shown as odds ratios (OR) with 95% confidence intervals (CI). PASW Statistics v18.0 was used for data analysis (SPSS Inc. released 2009 and PASW Statistics for Windows, Version 18.0, Chicago: SPSS Inc).

Table 1
Forensic collection methods of biological trace Y-chromosomal material by vaginal and cervical canal swabs and cervical canal brushes following consensual intercourse.

Collection method		Vaginal fornix swab		Cervical canal swab		Total
		+	−	+	−	*n* (%)
Cervical canal brush	+	15	19	9	25	34 (40.5)
	−	41	9	43	7	50 (59.5)
Total, *n* (%)		56 (66.6)	28 (33.3)	52 (61.9)	32 (38.1)	84

Table 2
Proportion of measurable Y-chromosomal material, according to the time from the consensual intercourse to the sample collection regarding the different sample collection methods.

Collection method	Time from the intercourse to the examination				Total
	<24 h	24 h–<48 h	48 h–<72 h	72 h-	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Vaginal fornix swab	23 (95.8)	16 (80.0)	7 (50.0)	10 (38.5)	56 (66.7)
Cervical canal swab	21 (87.5)	14 (70.0)	9 (64.3)	8 (30.8)	52 (61.9)
Cervical canal brush	21 (87.5)	12 (60.0)	9 (64.3)	8 (30.8)	50 (59.5)
Total	24 (28.6)	20 (23.8)	14 (16.7)	26 (31.0)	84 (100)

3. Results

3.1. Participant characteristics

The median age of 84 volunteers was 26.5 years (range 20–52). The median time from the consensual vaginal intercourse to the sample collection was 42.0 h (range 1.5–183.2).

3.2. Comparison of clinical forensic collection methods

A measurable amount of male DNA was detected from 56 (67.7%) vaginal fornix swabs, from 52 (61.9%) cervical canal swabs and from 50 (59.5%) cervical brush samples (Table 1). By combining all collecting techniques, 81% of the volunteers were Y-DNA positive.

Positive swab and brush samples are presented in Table 2 regarding the time from the study intercourse to the sample collection.

Up to 60 h, the conventional swab sampling techniques detected more Y-DNA positive samples compared to the brush technique. However, after 60 h the cervical canal brush sample technique showed its benefit by detecting 27.3% (6/22) more Y-DNA positive samples, which were Y-DNA negative by both conventional swab sampling techniques. When samples were collected less than 72 h following consensual intercourse, 2 (28.6%) brush samples out of 7 swab-negative samples were positive. When samples were collected over 72 h following intercourse, 4 (26.7%) brush samples out of 15 swab-negative samples were positive (Fig. 2).

In 9/84 (10.4%) volunteers Y-DNA was detected only in vaginal swabs while cervical swab and brush samples were negative. Male DNA was detected in 3/84 (3.6%) volunteers only in cervical swabs. From all the collected samples 16/84 (19%) volunteers were male DNA negative in every collection method. Of 58 volunteers studied within 72 h, 5/58 (8.6%) were Y-DNA negative, and 3/44 (6.8%) of volunteers were Y-DNA negative within 48 h.

By combining swab and brush techniques, 75% of volunteers were still Y-DNA positive between 72 and 144 h. After 144 h (6 days) Y-DNA was not detected.

The cut-off limit for possible DNA identification of the male was considered to be 0.01 ng/μl. The quantification and analysis resulted in measurable amounts of male DNA from which the mean quantities were 1.10 ng/μl (median 0.06 ng/μl; range 0.0005–23.3 ng/μl) from vaginal fornix swabs; 0.34 ng/μl (median 0.01 ng/μl; range 0.0011–10.6 ng/μl) from cervical swabs; and 0.16 ng/μl (median 0.02 ng/μl; range 0.0005–3.2 ng/μl) from cervical brushes (Fig. 3). No statistically significant differences were found when comparing the collection methods by cut-off limit of 0.01 ng/μl (Table 3).

The positivity of the sample was statistically significantly associated with the time from the consensual intercourse to the medical

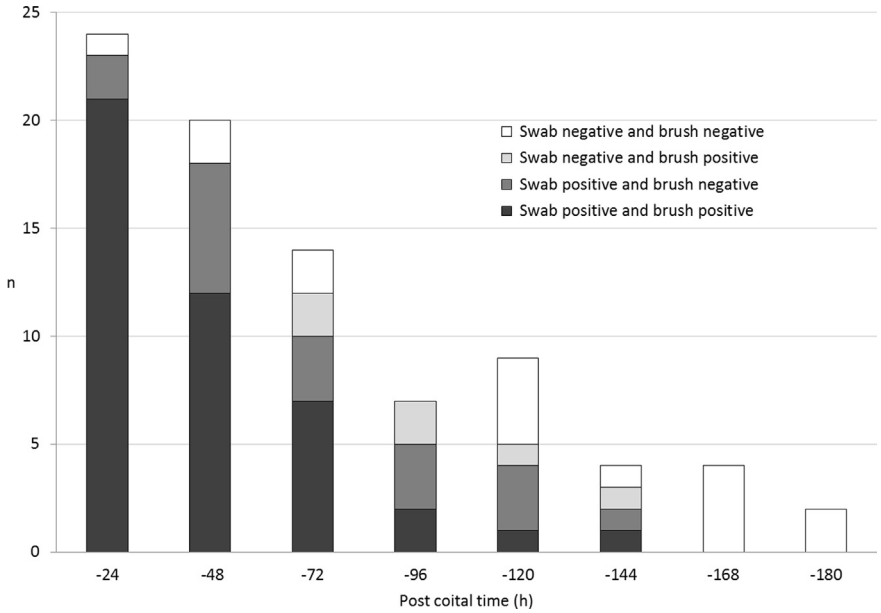

Fig. 2. Time interval from consensual sexual intercourse to the study examination and measurable male DNA's by forensic collection methods (swab samples from vaginal fornix and cervical canal; brush samples from cervical canal) per volunteer ($N = 84$).

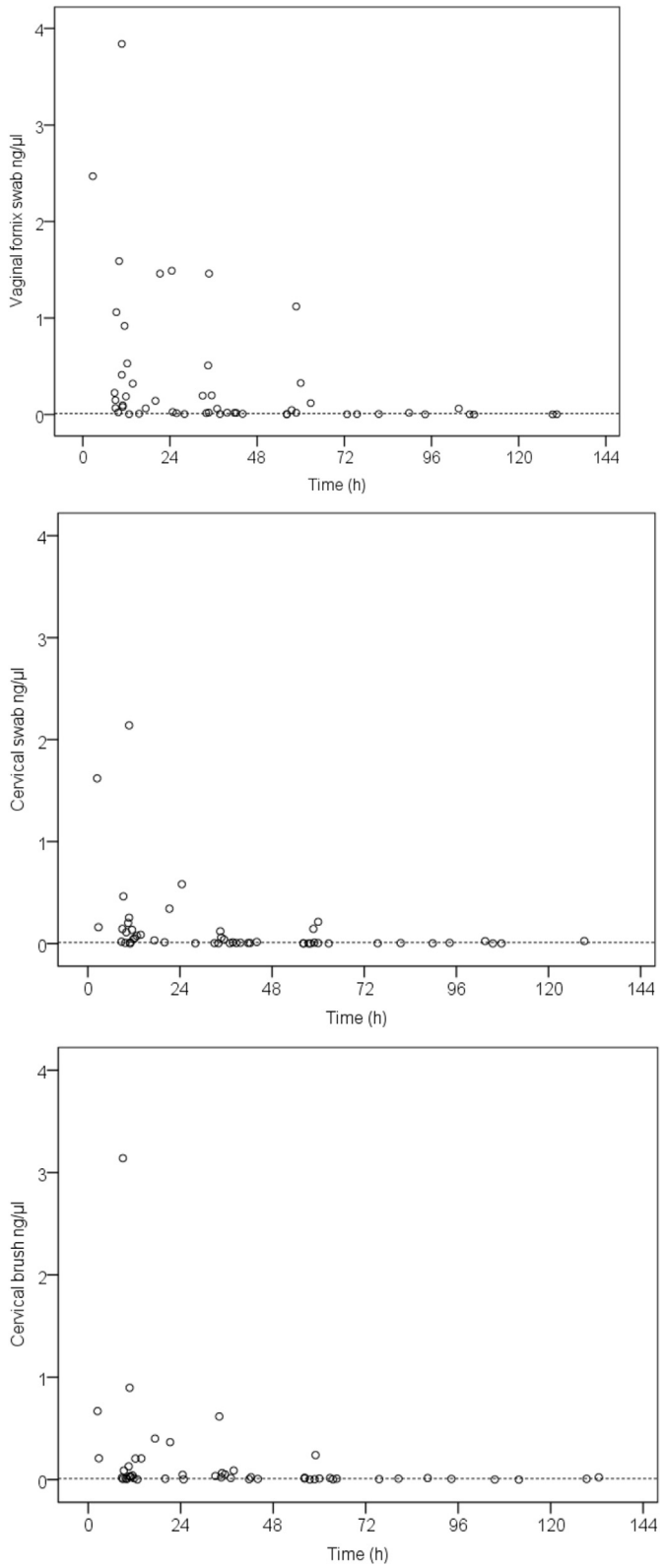

Fig. 3. Quantification of human male DNA according to time and <0.01 ng/µl cut-off limit.

examination. The positivity rate decreased by approximately 3% per hour (vaginal swabs OR = 0.97 (95% CI 0.96–0.98); cervical canal swabs OR = 0.97 (95% CI 0.96–0.99; and cervical brushes OR = 0.98 (95% CI 0.97–0.99).

Table 3
Comparing cervical brushes to cervical canal and vaginal fornix swabs regarding the possible cut-off limit for DNA identification being ≤0.01 ng/µl.

Cervical brush	Vaginal fornix swab		Cervical swab		Total
	≤0.01 ng/µl	>0.01 ng/µl	≤0.01 ng/µl	>0.01 ng/µl	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
≤0.01 ng/µl	37 (86.0)	14 (34.1)	43 (78.2)	8 (27.6)	51 (60.7)
>0.01 ng/µl	6 (14.0)	27 (65.9)	12 (21.8)	21 (72.4)	33 (39.3)
Total	43	41	55	29	84 (100)

3.3. Direct microscopic

Microscopy of slides was performed in 62/84 (73.8%) of the volunteers using two different microscopy techniques. Immediate phase-contrast microscopy performed in a subgroup of 39 volunteers by an experienced sperm microscopist (A.A.) detected spermatozoa in 7/39 (17.9%) volunteers; six (7.1%) with a few sperm and one (1.2%) with many spermatozoa per slide. Using the Kernechrout dye, spermatozoa with both body and tail were detected in 5/23 volunteers.

Altogether twelve (14.3%) volunteers had spermatozoa detected. Within 48 h, microscopy detected spermatozoa in 11/32 (34.3%) volunteers.

3.4. Post-coital activities, use of lubricants, coital durance, coital frequency, use of condom, and hormonal issues

Post-coital activities were reported in 21 volunteers, of which 16 (76.2%) swab samples and 12 (57.1%) brush samples remained positive.

Eight volunteers used lubricants during the studied intercourse. No statistically significant differences were found regarding the use of lubricants and Y-DNA positive samples.

Twenty-one volunteers did not use any hormonal products and five of them in the pre-ovulatory menstrual phase were positive in all collected samples. No statistically significant differences were found regarding the contraceptive method in use and Y-DNA positive samples.

Four volunteers used condoms for contraception. In two of them the quantity of Y-DNA was over 0.01 ng/µl.

The coital frequency per two weeks prior to the study intercourse was 0–3 times in 41 (48.8%) volunteers and 4 or more times in 43 (51.2%) volunteers. No statistically significant difference was found between coital duration, coital frequency during past two weeks prior to the study intercourse and the Y-DNA positivity.

4. Discussion

To our knowledge, this is the first study evaluating the value of a cervical canal PAP smear brush as a clinical forensic collection method for identification of Y-chromosomal material.

4.1. Cervical brush sample may provide additional evidence of a sexual contact after vaginal intercourse

Our study suggests a new forensic collection method; a cervical brush which may provide additional evidence in documenting the presence of Y-chromosomal material when compared to traditional vaginal and cervical swabs. When analyzing collected genital samples, the sensitivity of detection of Y-chromosomal material using Y-STR amplification methods is higher than finding spermatozoa in cytology,[4] as confirmed by our results showing 14.3% overall positivity by microscopy compared to 81.0% by PCR. Up to

60 h, the conventional swab sampling techniques detected more Y-DNA positive samples compared to the brush technique. However, after 60 h the cervical canal brush sample technique showed its benefit by detecting 27.3% (6/22) more male DNA positive samples, which were Y-DNA negative in both swab sampling techniques.

Despite reported consensual intercourse, 6.8% (3/44) volunteers were Y-DNA negative within 48 h. The possibility of negative semen samples following consensual intercourse in volunteers is an important factor in the legal evaluation of biological trace evidence. The negative forensic sample leads to the conclusion that it does not support nor exclude the possibility of vaginal penetration if a history of such is given.

This study shows that the risk of negative genital sample results after sexual intercourse increases by approximately 3% every hour. The cut-off time frame for vaginal and cervical forensic sample collection should be at least one week after suspected sexual assault in adults, which is consistent with earlier research.[4]

The vaginal fornix seems to be the best sampling site but it's not solely sufficient. Although an earlier study has a contradictory result,[6] the latest study found spermatozoa significantly better in the fornix posterior rather than in the external genitals or cervical canal.[1] In the present study, considering sampling from the cervical canal, the brush technique seems to be slightly better than the swab technique if the quantity of Y-DNA is considered. For the best possible biological trace evidence collection, all the collection sites are needed, as confirmed by our results. The benefits of the brush technique may be due to the collection of the remaining semen in cervical canal crypts by a brush compared with swabs as the brush attaches most of the cervical canal mucus to the brush.

4.2. Study limitations

The sample size, when divided into several post-coital time frames, was small, especially after 72 h and there is a need for larger studies. The risk of contamination should be taken into account, even though female researchers performed all collecting and sampling using standard techniques. This does not prevent secondary transfer of DNA from examination premises or forensic laboratory facilities. The quantified male DNA may also result from other sources than semen such as epithelial cells. Unfortunately, the women were not asked their opinion on possible ejaculation, which may explain part of the negative Y-DNA results.

5. Conclusions

The use of a cervical canal brush for collecting biological trace evidence from victims may provide additional evidence for criminal legal processes in suspected sexual violence cases. The gynecological forensic evidence collection should be considered for a week. It is important to understand the multiple factors influencing the low frequency of sperm-semen positivity, even following consensual intercourse.

Ethical approval

The study protocol was approved by the Ethics Committee (#R08018) of Pirkanmaa Hospital District, Finland.

Funding

None.

Conflict of interest

None.

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